BLISTER FORMATION AND SEPARATION OF THE EPIDERMIS FROM THE CORIUM IN LABORATORY ANIMALS*

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Common laboratory animals are not suited for the study of vesicant agents Blister formation does not occur in the skin of most hairy animals. Heretofore only the perfused frog (1), the duck (2, 3), the sole of the newborn pig (4) and the ear of the rabbit (after subcutaneous injection of coramine (5)) have been used for vesication. Even after death, the epidermis of most laboratory animals does not separate easily from the corium. Thus Blank and Miller's method of detaching the epidermis from the corium by negative pressure could be applied to human skin only. Attempts to separate the epidermis of the rabbit by this procedure have failed, because at a pressure where water was transmitted by the skin, the epidermis ruptured (6).

It appears that the difficulties which prevent separation of the epidermis from the corium in laboratory animals are caused by one or both of the following factors: 1. The epidermis of common laboratory animals is very thin, as compared with human epidermis. 2. The numerous and deep invaginations of the hair follicles anchor the epidermis to the deeper layers and prevent its separation (6, 7).

These difficulties may be overcome by pretreatment of the skin with a substance which causes hair loss together with acanthosis (7). Several such substances are known; their chemistry and pharmacologic action have been reported previously (7–11). The present study deals with the use of two of these compounds, namely the dimers of chloroprene and allyl laurate. After they have induced hair loss and acanthosis, the epidermis can be separated either post mortem by the vacuum method of Blank and Miller (6), or by blister formation *in vivo* by the common vesicants.

EXPERIMENTAL

I. Separation of the epidermis from the corium by negative pressure after death.

Mice and guinea pigs were depilated on the backs by local application of the chloroprene dimers or of allyl laurate.† Mice received a single application of 0.1 ml. dimer or of 0.1 ml. of 10% allyl laurate in absolute alcohol; guinea pigs were treated with 1 ml. of either of these depilatory compounds, used in full strength. The hair began to fall out 10 to 12 days after treatment with the dimers and 6

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† The chloroprene dimers were prepared as described elsewhere (8, 11); allyl laurate was obtained through courtesy of Dr. Madison Hunt, E. I. DuPont and Company.

to 8 days after allyl laurate. The hair loss was limited to the area of application. Signs of inflammation and crusting were marked after the use of allyl laurate. When these signs disappeared, the animals were killed, before they showed any visible signs of regeneration of hair. Immediately after death, the hairless skin was excised and water was drawn through it by negative pressure, according to the method of Blank and Miller (6). When visible blisters began to appear, the suction was discontinued. The skin was fixed in formalin and paraffin sections were prepared and stained with hematoxylin-eosin.

II. Blister formation in guinea pigs.

Guinea pigs were depilated with chloroprene dimers or with allyl laurate in the same way as was described above. It was best to treat the area between the ears and on the upper back with the depilatory compound, as these sites are the

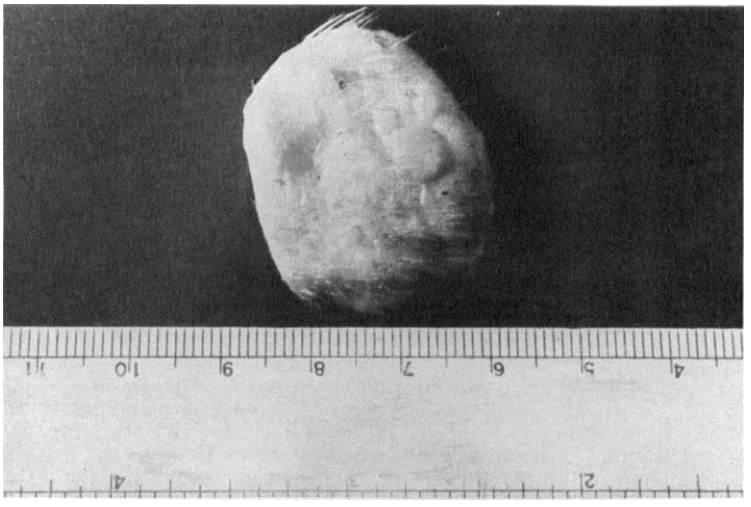

FIG. 1. Blisters in depilated guinea pig induced by negative pressure after death

least accessible to scratching which is provoked by the vesicant substance. For vesication we applied 0.3 ml. of 5 to 10% aqueous solutions of iodoacetic acid to the depilated areas. The animals were kept under observation for 4 hours. When gross signs of blister formation appeared, biopsy specimens were taken and routine formalin-fixed, paraffin-embedded sections were prepared and stained with hematoxylin-eosin.

RESULTS

I. Separation of the epidermis from the corium *post mortem*.

There was no difficulty in separating the epidermis from the corium in the depilated skin of the mouse and of the guinea pig with the suction method. Visible blisters began to appear in 30 to 40 minutes (Fig. 1). When the skin was exposed to low pressure for over an hour, the epidermis ruptured and the experiment had

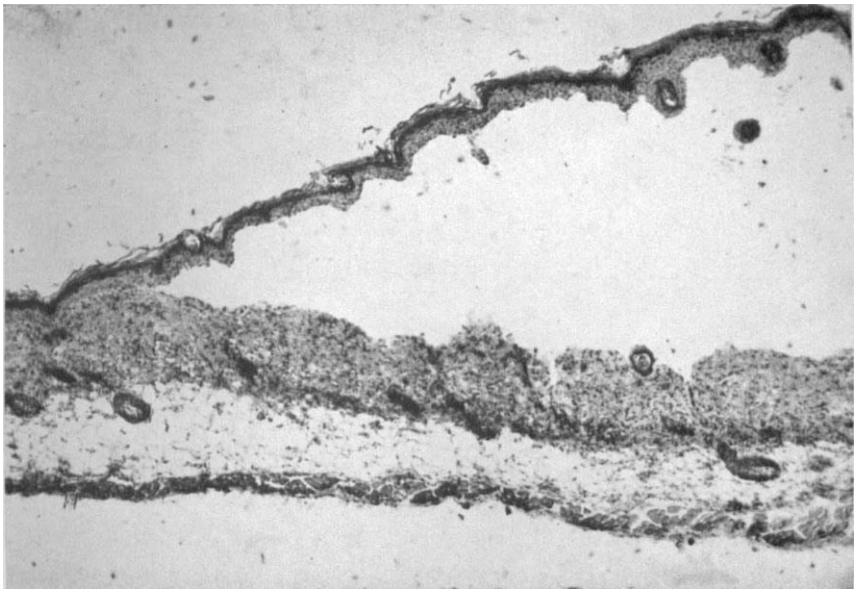

FIG. 2. Separation of guinea pig epidermis from corium in vacuo

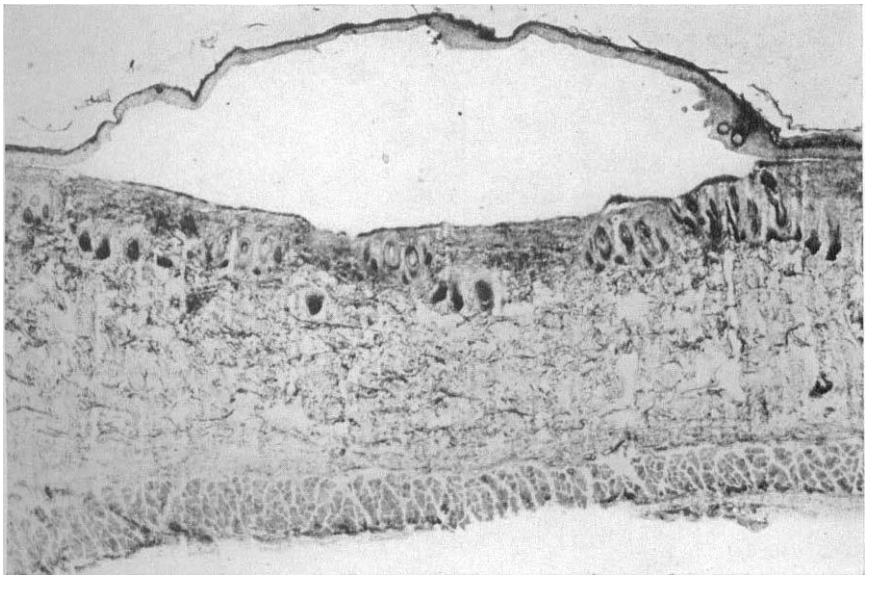

FIG. 3. Separation of mouse epidermis from corium in vacuo

to be discontinued. Separation of the epidermis from the corium did not succeed in a control group of normal animals with this method.

Histologic sections revealed separation of the epidermis from the corium at the epidermo-dermal junction. The epidermis was acanthotic from the effect

of the depilatory agent. The deeper type of hair follicle became detached from the epidermis and remained in the corium (Figs. 2 and 3).

II. Blister formation in guinea pigs *in vivo*.

One half to one hour after local application of iodoacetic acid to guinea pigs, the skin became red and swollen. The animals scratched violently and showed marked discomfort. At the end of three hours there were small blisters visible in the midst of eroded areas. Histologic sections showed separation at the epidermo-dermal junction with fibrin formation in the blister fluid (Fig. 4). Control animals, treated with the vesicant agent alone, showed no gross or microscopic

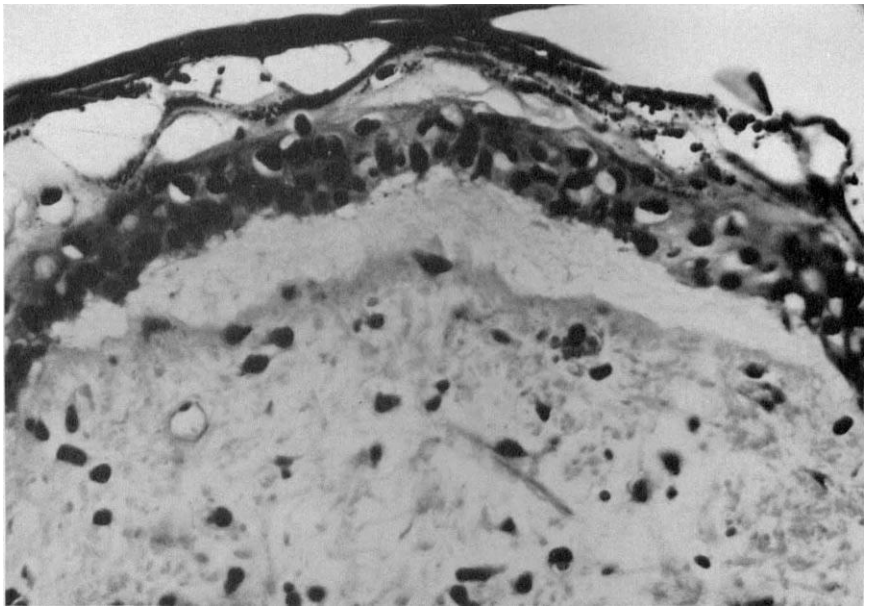

FIG. 4. Blister formation in depilated guinea pig after application of iodoacetic acid in vivo. Note accumulation of fibrin between epidermis and corium.

signs of vesication. The lesions produced by iodoacetic acid in the depilated animals healed within a few days. However, regrowth of hair on these sites was greatly retarded, as compared with the depilated areas which were not exposed to the vesicant agent.

We were unable to induce blisters in mice and rabbits with this method, but it is possible that they could be induced with other vesicant agents. A more complete investigation along these lines was beyond the scope of this study.

DISCUSSION

The success of this method is due partly to thickening of the epidermis. It would appear that such thickening gives a supportive ceiling for the vesicles which is lacking in normal animal skin, and prevents early rupture. In other

part, it would be due to the anchoring effect of the hair follicles. It is true that the deeper parts of the follicles still remain anchored, but they are weakened in their upper parts by the absence of the rigid, strong hair shaft and apparently also by lysis of the sheaths (Fig. 5). In any event, the follicles became ruptured subepidermally. The mechanism of such lysis (?) is not apparent.

As to the suction technic, this method makes the animal epidermis available for the first time in a presumably surviving (although hyperplastic) condition for *in vitro* studies. Experiments along these lines are in progress.

The vesicant action of iodoacetic acid in animals, previously described in human subjects (12), proves that this effect cannot be attributed to hypersensi-

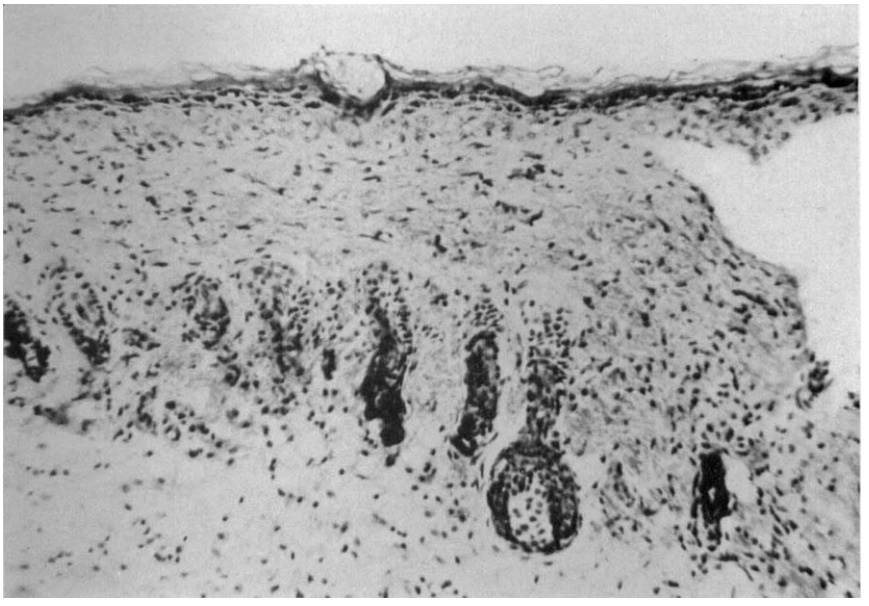

FIG. 5. Disappearance of upper portions of follicles in mouse skin from treatment with chloroprene dimer. At right, separation of epidermis from corium by suction.

tivity (13). Iodoacetic acid, a potent sulfhydryl inhibitor, apparently interferes with essential sulfhydryl enzymes in the same way as Lewisite. Its vesicant effect is probably due to a similar mechanism (1).

SUMMARY

A method is described of inducing blisters *in vivo* and *post mortem* in the skin of hairy laboratory animals.

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